Suitability of *S*-Phenyl Mercapturic Acid and *trans-trans*-Muconic Acid as Biomarkers for Exposure to Low Concentrations of Benzene

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Phenol is not reliable as a biomarker for exposure to benzene at concentrations below 5 ppm (8-hr time-weighted average [TWA]). *S*-Phenylmercapturic acid (S-PMA) and *trans-trans*-muconic acid (tt-MA), two minor urinary metabolites of benzene, have been proposed as biomarkers for low-level exposures. The aim of this study was to compare their suitability as biomarkers. S-PMA and tt-MA were determined in 434 urine samples collected from 188 workers in various settings in the petrochemical industry and from 52 control workers with no occupational exposure to benzene. Benzene concentrations in the breathing zone of the potentially exposed workers were assessed by personal air monitoring. Strong correlations were found between S-PMA and tt-MA concentrations in end-of-shift samples and between either of these parameters and airborne benzene concentrations. Exposure to 1 ppm benzene (8-hr TWA) leads to an average concentration in end-of-shift samples of 21 mol S-PMA and 1.5 mmol tt-MA per mol creatinine. Of an inhaled dose of benzene, on average 0.11% (range 0.05–0.26%) was excreted as S-PMA with an apparent elimination half-life of 9.1 (standard error [SE] 0.7) hr and 3.9% (range 1.9–7.3%) as tt-MA with a half-life of 5.0 (SE 0.5) hr. Due to its longer elimination half-life, S-PMA proved a more reliable biomarker than tt-MA for benzene exposures during 12-hr shifts. Specificity of S-PMA, but not tt-MA, was sufficient to discriminate between the 14 moderate smokers and the 38 nonsmokers from the control group. The mean urinary S-PMA was 1.71 (SE 0.27) in smokers and 0.94 (SE 0.15) mol/mol creatinine in nonsmokers ($p = 0.013$). The mean urinary tt-MA was 0.046 (SE 0.010) in smokers and 0.029 (SE 0.013) mmol/mol creatinine in nonsmokers ($p = 0.436$). The inferior specificity of tt-MA was due to relatively high background values of up to 0.56 mmol/mol creatinine, which may be found in nonexposed individuals and limits the use of tt-MA to concentrations of benzene over 1 ppm (8-hr TWA). We conclude that S-PMA is superior to tt-MA as a biomarker for low-level benzene exposures because it is more specific, enabling reliable determination of benzene exposures down to 0.3 ppm (8-hr TWA), and because its longer half-life makes it more suited for biological monitoring of operators working in shifts longer than 8 hr. — Environ Health Perspect 104(Suppl 6):1151–1157 (1996)

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Introduction

Long-term occupational exposure to high concentrations of benzene (100–500 ppm) has been associated with potential adverse health effects, including leukemogenesis (*1*). Therefore, exposure to benzene should be minimized. From the late 1980s on, proposals were made and adopted in many countries to lower the occupational exposure limit value for benzene from 10 to 1 ppm (8-hr time-weighted average [TWA]). In some countries, even lower values have been adopted (e.g., 0.5 ppm in Sweden) or proposed (0.3 ppm in the United States) (*2,3*). Urinary phenol is not suitable as a biomarker for benzene exposure at levels below approximately 5 ppm due to its high and variable background caused by endogenous production of phenol as well as dietary and environmental exposure to phenol. Thus, new biological monitoring techniques were needed to evaluate the effectiveness of control measures. Two minor urinary metabolites of benzene, *trans-trans*-muconic acid ([tt-MA], 2,4-hexadienedioic acid) (Figure 1) and *S*-phenylmercapturic acid ([S-PMA], *N*-acetyl-*S*-phenyl-L-cysteine) (Figure 1), were proposed to replace phenol in biological monitoring of exposure to benzene (*4,5*). Since only S-PMA seemed to enable detection of exposure to benzene at levels below 1 ppm, the method of Stommel and co-workers (*5*) for determination of urinary S-PMA was modified in our laboratory to be applicable for routine biological monitoring. This S-PMA test was validated in 12 separate studies in various chemical manufacturing plants, oil refineries, and natural gas production installations and proved a reliable and sensitive biomarker capable of measuring 8-hr TWA benzene exposures of 0.3 ppm and higher (*6*). In the past few years, the method by Inoue and coworkers for assessment of tt-MA has also been modified by several investigators, and a sensitivity as low as 1 ppm (8-hr TWA) has been reported (*7–9*). The determination of S-PMA requires gas chromatography–mass spectrometry (GC–MS), whereas the determination of tt-MA is based on high performance liquid chromatography (HPLC) analysis, which is in general more readily available in a laboratory and easier to operate. We measured tt-MA and S-PMA in workers with potential occupational exposure to benzene and control workers to compare the suitability of both biomarkers to detect low levels of benzene exposure.

Materials and Methods

Chemicals

S-Phenylmercapturic acid (> 98%), tt-MA (> 98%), and acetic acid (> 99.7%) were purchased from Janssen Chimica (Goirle, the Netherlands). Ethyl acetate (> 99.5%, HPLC grade), dichloromethane (coffee grade), methanol (> 99.9%, capillary GC grade) and purified water (HPLC grade) were obtained from J.T. Baker Chemicals (Deventer, the Netherlands). Hydrochloric acid gas (technical grade) was purchased from Hoekloos (Schiedam, the Netherlands). *S*-[Pentadeuterophenyl]mercapturic acid

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Abbreviations used: AUC, area under the curve; d_5-S-PMA, *S*-[pentadeuterophenyl]mercapturic acid; EKA, exposure equivalent for carcinogenic substances; GC, gas chromatography; HPLC, high performance liquid chromatography; k_e, elimination rate constant; MS, mass spectrometry; S-PMA, *S*-phenylmercapturic acid; $t_{1/2}$, elimination half-life; tt-MA, *trans-trans*-muconic acid; TWA, time-weighted average.

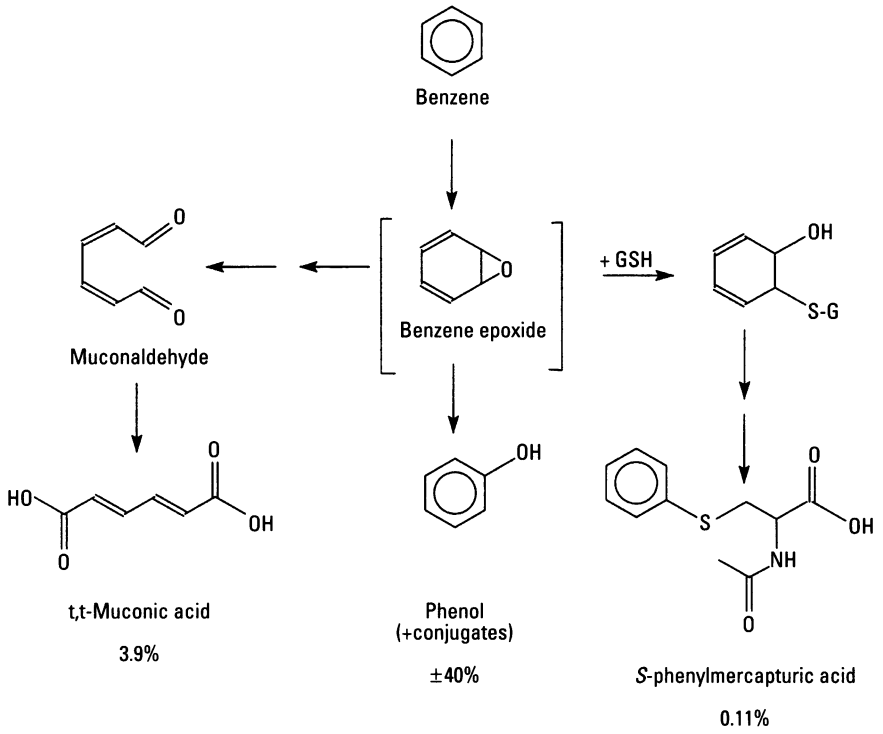

Figure 1. Some metabolic pathways of benzene and urinary excretion of benzene metabolites. Indicated are the formation of tt-MA, phenol, and S-PMA and the percentage of the retained dose excreted into the urine after inhalation of benzene vapors for each of these metabolites.

(d_5-S-PMA) was synthesized from d_5-aniline as described previously (*10*).

Study Populations and Collections of Urinary and Airborne Samples

Urinary tt-MA and S-PMA were determined in 52 employees without potential exposure to benzene for the estimation of normal values. This control group comprised 14 smokers and 38 nonsmokers.

For the comparison of the biomarkers, 434 urine samples were collected between 1992 and 1994 from 188 workers with potential exposure to benzene during manufacturing and maintenance operations in three off-shore natural-gas production installations, in two refineries with aromatics plants, in various chemical plants, and during loading of road tankers with gasoline. The numbers of samples collected, the number of workers involved, and the duration of the shifts are summarized in Table 1.

Urine samples were collected in polyethylene bottles and acidified to pH 2 by addition of 6 M hydrochloric acid. Stability studies of S-PMA and tt-MA in urine had shown that concentrations did not change under these conditions for at least a month if stored at room temperature or 4°C (*10*). The samples were transported to the Shell Biomedical Laboratory, Rotterdam, for determination of urinary S-PMA, tt-MA and creatinine. Urinary creatinine was previously shown to provide a good adjustment for the variations in concentration of spot urine samples due to differences in fluid intake among workers (*6,10*). For many workers with potential exposure to benzene, personal air sampling of benzene in the breathing zone was performed by local staff using 3M gas diffusion badges or charcoal tubes. The absorbed benzene vapors were desorbed by carbon disulfide and the solution was analyzed using a gas chromatographic method with flame ionization detection. Sampling and analysis were according to standard methods published by the National Institute for Occupational Safety and Health (*11*). No personal air monitoring data were available from the workers involved in the loading of road tankers, the operators in one of the oil refineries, or the workers in one of the chemical plants. From some of the workers in the other chemical plants, no personal air monitoring data were available because biological monitoring was applied after accidental potential exposure to benzene (Table 1).

Determination of Urinary Metabolites

For the determination of tt-MA, the method by Ducos et al. (*7,8*) was modified because the recovery of tt-MA appeared to be dependent on the urinary pH with some brands of the quaternary ammonium ion exchange resin (SAX columns) used. Alkalization of the samples to pH 7 to 10 prior to extraction gave optimum recoveries, which were invariably over 95% (10). An aliquot of 1.00 ml of alkaline urine was applied to a SAX column, which was subsequently washed with 3 ml 1% (v/v) aqueous acetic acid. The tt-MA was then eluted

Table 1. Summary of benzene exposure monitoring studies in various petrochemical industries.

	Urinary metabolites						Airborne benzene		
	tt-MA, mmol/mol creatinine		S-PMA, µmol/mol creatinine		No. of workers, measurements			No. of workers, measurements	
Operation	Start of shift	End of shift	Start of shift	End of shift	8-hr shift	12-hr shift	Concentration, mg/m³, 8-hr TWA	8-hr shift	12-hr shift
Natural gas production platforms	< 0.01–2.6	< 0.01–9.92	< 0.5–235	< 0.5–378	–	24 (70)	< 0.1–19.2	–	24 (59)
Chemical manufacturing	< 0.01–0.06	< 0.01–31.3	0.9–21.8	< 1–1096	130 (226)	4 (22)	< 0.01–100	50 (102)	4 (22)
Oil refineries with aromatics plants	–	0.008–1.20	–	0.9–46.4	16 (22)	–	0.11–3.30	8 (14)	–
Road tanker loading	0.008–0.33	0.009–0.83	< 0.5–6.64	0.5–8.0	14 (42)	–	–	–	–

with 4 ml 10% (v/v) aqueous acetic acid into calibrated tubes and the volume made up to 5.0 ml with purified water. Aliquots (20 µl) of this solution were analyzed by HPLC with UV (λ=259 nm) detection (Hewlett Packard 1084B) at 20°C using a 100 × 2.1 mm stainless steel column with Spherisorb 5 ODS-2 as immobile phase and 20% (v/v) methanol in 1% (v/v) aqueous acetic acid as mobile phase (flow rate 1 ml/min). For calibration, a series of tt-MA standards prepared in fresh control urine samples were analyzed together with the urine samples. A more effective clean-up was an adventitious effect of the alkalization of the samples that lowered the detection limit to 70 nM. The coefficient of variation of replicate analyses ($n = 10$) was 3.2 and 0.4% at spiked concentrations of 4.51 and 39.1 µM, respectively. The reproducibility was tested by analysis of a set of identical samples in a series ($n = 6$) of runs. The coefficient of variation was 11 and 2.7% for samples with a spiked concentration of 7.82 and 39.2 µM, respectively. With this procedure, up to 40 urine samples could be analyzed in 1 day.

For the determination of S-PMA, d_5-S-PMA was added to an aliquot of the acidified urine sample, which was subsequently extracted with ethyl acetate. After evaporation of the solvent, the residue was methylated using a solution of 1.0 M hydrochloric acid in methanol. The organic phase was evaporated and the residue dissolved in dichloromethane and analyzed by GC–MA as described previously (*6,10*). The use of d_5-S-PMA as internal standard allowed a limit of detection of 4 nM. A series of urine samples was pooled, spiked with S-PMA at two different concentrations, acidified, and stored frozen at –20°C. In all runs, a set of samples from this urine pool was analyzed to check the reproducibility of the method. The coefficients of variation between different runs ($n = 42$) were 8.9 and 6.1% for the pools with 118 and 437 nM S-PMA, respectively. The coefficients of variation of replicate analyses ($n = 10$) were 2.5 and 3.5% at spiked concentrations of 55 and 211 nM S-PMA, respectively. With this procedure, up to 30 urine samples could be analyzed in one day.

Creatinine was determined as described previously (*6*).

Calculations and Statistics

The cumulative excretions of tt-MA and S-PMA were estimated from the area under the curve (AUC) from urinary excretion vs time plots and were used to estimate the percentages of the respiratory benzene dose excreted as tt-MA and S-PMA into the urine. AUCs were calculated using the linear trapezoidal rule in the absorption phases and the logarithmic trapezoidal rule in the elimination phases as described previously (*6,10*). Apparent urinary elimination rate constants (k_e) for S-PMA and tt-MA were calculated from workers who had provided urine samples at the beginning and end of a shift on at least two consecutive days. For the calculation of each k_e, it was assumed that the absorption was completed and that first-order elimination kinetics were obeyed during the collection period. In some cases, the absorption phase was not yet completed at the end of the shift. In these cases, k_e was calculated from the decline in metabolite during the same shift but only if there was no detectable exposure to benzene. For most workers, values for k_e could be calculated more than once. In these cases the separate values were averaged. From the averaged values, the corresponding half-life ($t_{1/2}$) was calculated for each worker using the following formula: $t_{1/2} = -\ln(2)/k_e$ (*6,10*).

For all statistical calculations, the mainframe version of the SAS software package, version 6.07 (SAS Institute, Cary, NC), was used. For S-PMA and tt-MA results below the calculated value for the limit of detection of the respective methods, half of this value was substituted.

Results

Relation between Urinary tt-MA and S-PMA

The tt-MA concentrations were plotted against the S-PMA concentration in the same urinary sample, both following logarithmic transformation, for all 188 workers in the study (Figure 2). For many workers, more than one set of values was determined. In these cases the set of values corresponding to the highest airborne benzene concentration, or the set with the highest value of S-PMA if no air measurements were performed, was chosen. A statistically highly significant correlation ($r = 0.79$, $p < 0.0001$) between tt-MA and S-PMA was found. This correlation is described by the following equation: log (tt-MA [mmol/mol creatinine]) = 0.824 (SE 0.048) × log (S-PMA [mol/mol creatinine]) – 0.993 (SE 0.062). Nevertheless, some deviations were observed. In some cases where no exposure to benzene had occurred, relatively high concentrations of tt-MA (up to 0.56 mmol/mol creatinine) were measured in the urine (with no concomitant S-PMA, concentration ≤ 0.5 mol/mol creatinine).

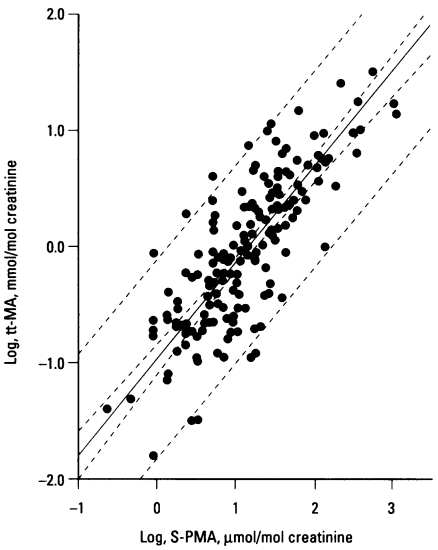

Figure 2. Relationship between urinary concentrations of tt-MA and S-PMA in 188 workers exposed to benzene. The solid line represents the regression line, and the broken lines represent the 95% tolerance limits of the confidence interval for individual (outer lines) and group (inner lines) results.

Urinary Excretion Kinetics of tt-MA and S-PMA

Typical urinary excretion vs time plots from two workers who were biologically monitored for 3 consecutive days are shown in Figure 3. The first worker (Figure 3A) worked 8-hr shifts and was exposed to very low concentrations of benzene (0.6 mg/m^3, 8-hr TWA) during the first 2 working days and to slightly higher concentrations on the third working day (2.4 mg/m^3, 8-hr TWA). The second worker (Figure 3B) worked 12-hr shifts and was exposed to higher concentrations of benzene: 1.95, 5.20, and 3.25 mg/m^3 (8-hr TWA) on the first, second, and third day, respectively. As shown in both graphs, excretion of tt-MA and S-PMA obviously followed the same pattern, and the excretion of both metabolites reflected the exposure to benzene. These graphs also indicate that the urinary excretion half-life was longer for S-PMA than for tt-MA and that tt-MA returned to baseline values at the beginning of the next shift after exposure to benzene below approximately 1 ppm, whereas S-PMA tended to accumulate.

For 27 workers who provided pre- and post-shift urine samples on at least 2 consecutive days, values for the apparent urinary

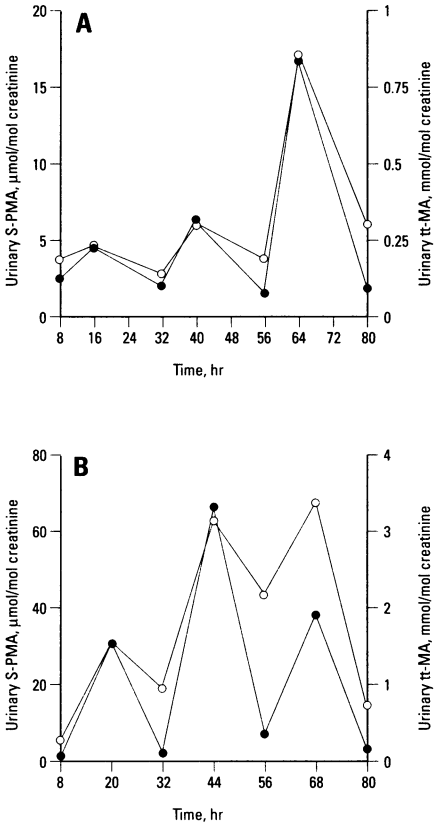

Figure 3. Urinary excretion of S-PMA (○) and tt-MA (●) in two operators working in 8-hr shifts (*A*) and 12-hr shifts (*B*) during 3 consecutive days.

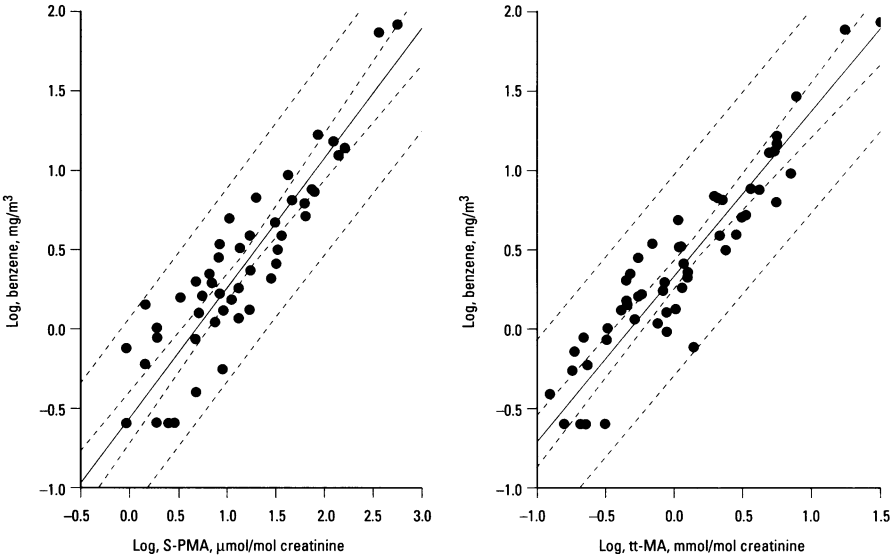

Figure 4. Relationship between respiratory 8-hr TWA exposure to benzene and concentrations of S-PMA and tt-MA in urinary samples collected at the end of an 8-hr shift from 58 operators working in chemical manufacturing plants and oil refineries with aromatics plants. The solid lines represent the regression lines, and the broken lines represent the 95% tolerance limits of the confidence interval for individual (outer lines) and group (inner lines) results. The regression line for S-PMA is given by log (benzene [mg/m^3, 8-hr TWA]) = 0.782 (SE 0.064) × log (S-PMA [µmol/mol creatinine]) − 0.518 (SE 0.081); r = 0.885. The regression line for tt-MA is given by log (benzene [mg/m^3, 8-hr TWA]) = 1.04 (SE 0.072) × log (tt-MA [mmol/mol creatinine]) + 0.320 (SE 0.040); r = 0.888.

excretion constants could be calculated from 38 and 52 data points for S-PMA and tt-MA, respectively. The median k_e was 0.077 liters/hr for S-PMA and 0.136 liters/hr for tt-MA. These values correspond to apparent elimination half-lives of 9.1 (SE 0.7) and 5.0 (SE 0.5) hr, respectively. For 14 workers, sufficient personal air monitoring and biological monitoring data were collected to allow estimations of the urinary excretions of tt-MA and S-PMA as percentages of the dose of inhaled benzene. The average percentage of the dose that excreted as tt-MA was 3.9% (range 1.9–7.3%) and excreted as S-PMA was 0.11% (range 0.05–0.26%).

Relation between Airborne Benzene and Urinary Excretion of tt-MA and S-PMA

The relationship between airborne benzene exposures (8-hr TWA) and urinary S-PMA and tt-MA concentrations in samples collected at the end of the shift from the 58 workers on 8-hr shift duty are shown in Figure 4. The actual working hours for these operators were close to 8 hr. Data from workers who had worn respiratory protection during the monitoring period were excluded from the statistics. In cases in which more than one data point had been generated for a worker, the data point with the highest airborne benzene level was used for the linear regression analysis after logarithmic transformation of the airborne benzene concentration data and the urinary metabolite data. Highly significant correlations ($p < 0.0001$) were found for both S-PMA and tt-MA.

From the regression lines, it was calculated that the average concentration in a urine sample collected at the end of the shift following an 8-hr TWA benzene exposure to 1 ppm (3.25 mg/m^3), would be 21 µmol S-PMA/mol creatinine and 1.5 mmol tt-MA/mol creatinine. The corresponding 95% tolerance limits of the confidence interval for the group means were 17 to 27 µmol S-PMA/mol creatinine and 1.3 to 1.8 mmol tt-MA/mol creatinine, respectively.

A separate regression analysis was made using the data collected from the 28 operators working 12-hr shifts (Figure 5). Again, only one set of data was included for each worker, and data collected while respiratory protection was worn were excluded.

Urinary tt-MA and S-PMA Concentrations in the Control Population

The 38 nonsmokers in the control group had a mean urinary tt-MA concentration of 0.029 (SE 0.013), while the 14 smokers had a concentration of 0.046 (SE 0.013) mmol/mol creatinine. The mean S-PMA concentration in the 38 nonsmokers was 0.94 (SE 0.15) and in the 14 smokers 1.71 (SE 0.27) µmol/mol creatinine. The difference in tt-MA between smokers and nonsmokers was not statistically different ($p = 0.436$), but the difference in S-PMA was ($p = 0.013$, two-tailed Student's t-test for unpaired data). S-PMA was present at detectable concentrations in the urine of all 14 smokers and in urine samples of 20 of the 38 nonsmokers. Urinary concentrations of tt-MA in 3 smokers and 29 nonsmokers were below the detection limit.

Discussion

Lowering the occupational limit values for benzene has made urinary phenol obsolete as a biomarker for benzene exposure. Two minor metabolites of benzene (Figure 1) have been proposed to replace phenol as a biomarker for benzene exposure: S-PMA (*5,6*) and tt-MA (*4,7*). The suitability of these new biomarkers in terms of specificity, sensitivity, and ease of operation was

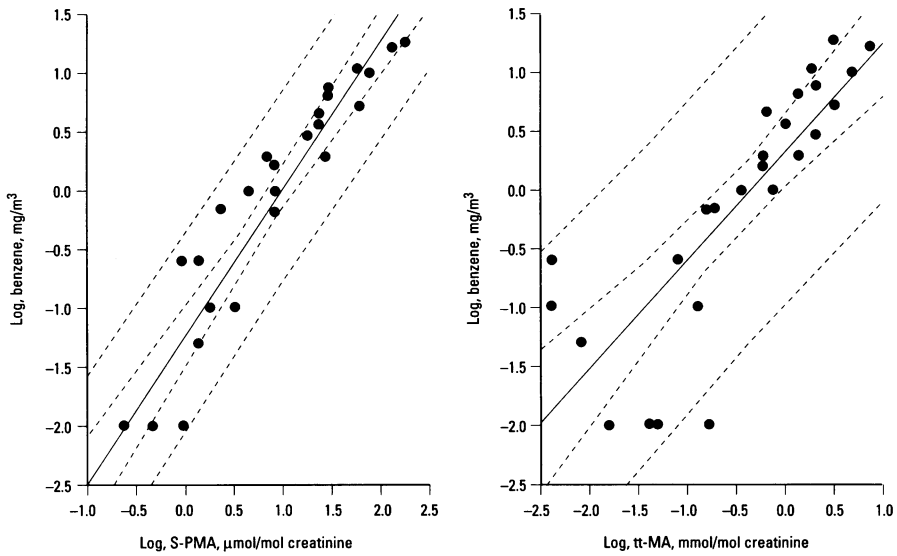

Figure 5. Relationship between respiratory 12-hr TWA exposure to benzene and concentrations of S-PMA and tt-MA in urinary samples collected at the end of a 12-hr shift from 28 operators working in off-shore natural gas production platforms and in chemical manufacturing. The solid lines represent the regression lines and the broken lines represent the 95% tolerance limits of the confidence interval for individual (outer lines) and group (inner lines) results. The regression line for S-PMA is given by log (benzene [mg/m^3, 12-hr TWA]) = 1.243 (SE 0.094) × log (S-PMA [µmol/mol creatinine]) − 1.24 (SE 0.11); r = 0.937. The regression line for tt-MA is given by log (benzene [mg/m^3, 12-hr TWA]) = 0.92 (SE 0.13) × log (tt-MA [mmol/mol creatinine]) + 0.33 (SE 0.14); r = 0.819.

investigated by analyzing 434 urine samples collected from 188 workers with potential exposure to benzene and 52 control workers.

The method for determining S-PMA in urine using *S*-benzylmercapturic acid as an internal standard was validated in a series of studies in workers in petrochemical industries (*6*). The use of d_5-S-PMA as an internal standard in the present study decreased interference of matrix effects, which resulted in a 4 nM limit of detection. Another advantage of d_5-S-PMA over *S*-benzylmercapturic acid is that the latter may also be found in trace amounts in the urine of workers exposed to toluene (*10,12*). Overall, the present studies confirmed the previous observations that S-PMA is a reliable biomarker for exposure to concentrations of benzene well below 1 ppm (8-hr TWA).

The method for determining tt-MA as described by Ducos and co-workers (*7,8*) gave irreproducible results with some brands of quaternary ammonium exchange columns because the recovery of tt-MA from these columns appeared dependent on the pH of the urine samples. This problem could be solved by adjusting the pH of the samples to a pH of 7 to 10 with sodium hydroxide prior to extraction. The solid-phase extraction was apparently more efficient at higher pH, which resulted in cleaner extracts and a lower limit of detection (70 nM). Overall, the method proved simple, straightforward, and robust, which resulted in small intrarun as well as interrun variation coefficients.

In our previous studies we did not find a statistically significant difference in urinary S-PMA in smokers compared with that in nonsmokers (*6*). Due to the increased sensitivity of the S-PMA determination, benzene uptake from cigarette smoking was now detectable in all 14 smokers from the control group. In 20 of the 38 nonsmokers of the control group S-PMA could also be detected, albeit in much lower concentrations. The difference in urinary S-PMA of the 14 smokers (1.71 µmol/mol creatinine, SE 0.27) and the 38 nonsmokers (0.94 µmol/mol creatinine, SE 0.15) from the control group was statistically significant. In some smokers and most nonsmokers the concentrations of tt-MA were below the detection limit and there was no statistically significant difference in urinary tt-MA in the smokers (0.046 mmol/mol creatinine, SE 0.010) and nonsmokers (0.029 mmol/mol creatinine, SE 0.013). This is in apparent contrast to two recent studies in which significant differences were observed between mean urinary tt-MA concentrations in 35 smokers and 23 nonsmokers (0.15 vs 0.11 mmol/mol creatinine) (*9*) and between 42 smokers and 42 nonsmokers (0.23 vs 0.07 mmol/mol creatinine) (*13*). From the absolute values, however, it may be concluded that the smokers in these two studies consumed much larger quantities of cigarettes than the moderate smokers in our control group. This, of course, makes the difference found in S-PMA more prominent. The mean background values in the nonsmokers in these studies are high compared to our results. The investigators contributed these backgrounds to environmental pollution. However, they might be due to matrix interference. In a recent study that used a GC–MS method for the determination of tt-MA to avoid interference by other compounds from the urinary matrix with tt-MA, the background was only 0.043 mmol/mol creatinine (SE 0.04) in nonsmokers versus 0.071 mmol tt-MA/mol creatinine (SE 0.09) in smokers (*14*).

Relatively high background values of tt-MA in individuals without exposure to benzene have repeatedly been reported (*8,13,15,16*). This background is partly due to compounds in the urine that coelute with tt-MA in the HPLC analysis. Bartczak and co-workers (*17*) showed that modifying the HPLC-based assay of tt-MA, as described by Ducos et al. (*8*), by using a diode array detector increased the specificity by identifying false positives. However, the diode array detector is less sensitive (limit of detection 0.6 µM) and, moreover, GC analysis showed that in some samples peaks were falsely identified as tt-MA even with the diode array detector, and in other samples the concentration of tt-MA was overestimated (*17*). Recently, a GC–MS-based assay for the determination of tt-MA with a similar detection limit as our HPLC method (70 nM) was reported (*14*). Although this method is highly specific for tt-MA, some tt-MA could still be detected in nonsmoking individuals who were not occupationally exposed to benzene (*14*). These traces of tt-MA may be due to consumption of sorbic acid, which is used as a preservative in many foodstuffs and is metabolized to tt-MA (*7,14,18*).

Based on spot samples collected from 27 workers on two or more consecutive days, the average apparent urinary elimination half-life ($t_{1/2}$) for S-PMA was estimated at 9.1 hr (SE 0.7), which is very similar to the $t_{1/2}$ of 9.0 hr (SE 0.8) previously reported (*6*). For tt-MA, the estimated $t_{1/2}$ for tt-MA was 5.0 hr (SE 0.5), which is in line with the observation that tt-MA and phenol have similar half-lives (7). The average percentage of the doses of inhaled benzene that were

excreted in the urine as S-PMA was 3.9% (range 1.9–7.3%), which is very similar to our previous findings (*6*). For tt-MA a value of 0.11% (range 0.05–0.26%) was found, which is about twice the only value reported thus far (1.9%) (*4*). This difference is partly explained by the lower respiration rate of 0.9 liters/hr used for the estimation by Inoue and co-workers compared with the rate we used (1.25 liters/hr). The rather wide ranges for urinary excretion as a percentage of the dose for both tt-MA and S-PMA excretion may be due partly to the fact that the calculations are based on spot samples, which carries the inherent risk of under- and overestimation of AUC.

A good correlation between tt-MA and S-PMA (Figure 2) was found, and the values for S-PMA and tt-MA that were recently determined in 26 car mechanics (*19*) fit this correlation well. Also good correlations were found between either S-PMA or tt-MA and airborne benzene concentrations (Figures 4, 5). The confidence intervals in Figure 4 as well as the correlation coefficients for the regression lines indicate that the reliability of tt-MA and S-PMA as biomarkers for benzene exposure during 8-hr shifts is approximately identical. Figure 5 shows that the reliability of tt-MA as a biomarker for benzene exposure during 12-hr shifts is much less than for S-PMA, which is fully due to its shorter elimination half-life. From the correlation found between both S-PMA and tt-MA with the airborne concentration of benzene, it follows that exposure to 1 ppm benzene (8-hr TWA) will lead to an average excretion of 21 μmol S-PMA and 1.5 mmol tt-MA/mol creatinine in urine samples collected at the end of the shift. These values seem to be confirmed by the regression equation in Figure 2, from which it can be calculated that 21 μmol S-PMA/mol creatinine corresponds to 1.3 mmol tt-MA/mol creatinine. This value for tt-MA is slightly less than the value of 1.5 mmol/mol creatinine. This is explained by the fact that values from workers on 12-hr shift duty, which in general will be lower because the half-life of tt-MA is much shorter than the half life of S-PMA, are also included in the correlation between tt-MA and S-PMA. The value of 21 μmol/mol creatinine for S-PMA also agrees well with the findings from our previous studies, where a value of 22 mol/mol creatinine was found (*6*), which is now accepted as the exposure equivalent for carcinogenic substances (EKA) at 1 ppm (8-hr TWA) (*20*). The value of 1.5 mmol/mol creatinine for tt-MA is higher than the values of 0.07 mM and 1.1 mmol/mol creatinine that were found corresponding with exposure to 1 ppm benzene (8-hr TWA) in two other studies but well in line with the EKA for tt-MA at 1 ppm benzene (8-hr TWA) of 0.014 mM (*20*), which corresponds to 1.6 mmol/mol creatinine, assuming an average urinary creatinine concentration of 1 g/liter.

In the present study the suitability of tt-MA and S-PMA as biomarkers for exposure to low concentrations of benzene was compared. Although the analytical sensitivity of the S-PMA assay is much higher (detection limit of 4 nM) than that of tt-MA (detection limit of 70 nM), tt-MA potentially is a more sensitive biomarker than S-PMA because 3.9% on average will be excreted as tt-MA and only 0.11% as S-PMA following inhalation exposure to low concentrations of benzene. Another reason to prefer the HPLC-based tt-MA assay over the GC–MS-based S-PMA assay might be that the tt-MA assay is easier to perform and enables a larger sample throughput than GC–MS and also that HPLC is more readily available than GC–MS in many cases. Due to the rather expensive solid-phase extraction, the analysis of tt-MA approximately equals the analysis of S-PMA in terms of costs per sample. An important drawback, however, of the HPLC-based tt-MA assay is its lack of specificity, which precludes reliable detection of benzene exposure below approximately 1 ppm (8-hr TWA), whereas S-PMA allows detection of concentrations as low as 0.3 ppm (8-hr TWA). This disadvantage of the HPLC-based tt-MA assay may to a great extent be overcome by using a diode array detector, but this will result in a major loss of sensitivity (*17*). Another option is to apply GC–MS for determination of tt-MA. However, the methods now developed are more cumbersome and time-consuming than the GC–MS-based S-PMA assay (*14,17*). Also, the specificity problem caused by the conversion of dietary sorbic acid to tt-MA will remain. Since the longer urinary half-life of S-PMA compared with that of tt-MA also makes S-PMA a more reliable biomarker for workers in shifts longer than 8 hr, we conclude that S-PMA is superior to tt-MA as a biomarker for exposure to low concentrations of benzene.

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